PROTOCOL FOR THE HISTOLOGICAL AND IMMUNOHISTOCHEMICAL PROCESSING OF THE CORTICAL SLICES

I. Slice resection

1. Run 4 slices. Each slice should be kept in separate vial.
2. Take the slices from the freezing solution
3. Rinse in PB for 10 min.
4. Fix in 4% paraformaldehyde for 4h (overnight for vibrotoming)
5. Put in 30% Sucrose overnight in refrigerator
6. Make a platform from 30% Sucrose on the freezing microtome
7. Trim the platform to make it flat
8. Place the slice on small piece of a glass slide **with the appropriate surface faced to the glass! This surface will be faced up on the microtome stage.**
9. Carefully drop a slide with the slice on the platform and weight a few seconds, then slide out the glass from the slice
10. Set microtome to 20 μm increments and position the knife as close as possible to the surface of the slice
11. **The crucial point is not to loose any tissue from the surface!**
12. **Without changing the microtome settings** cut 5 consecutive sections 60 μm thick and collect sections from the same slices in the same vials filled with PB (10ml)
13. Discard the rest of the slice
14. Section #5 mount on the gelatin-coated slide and stain for Nissl

II. Immunohistochemical protocol for BIOCYTIN only

1. 1 % H_2O_2 (**330 μl of 30% H_2O_2 for 10ml of PB**) RT (on slow rotation) before all bubbles are gone from the sections (4-5 hrs or more)
2. BS (1ml/vial) 24 h at 4^0 (on slow rotation)
3. Rinse in PB 1x10 min
4. ABC (Prepare 30 min prior to incubation) (1ml/vial) 4 h at RT (on slow rotation)
5. Rinse in PB 3x10 min at RT (on shaker)
6. **NI-DAB Reaction for 20 ml of DAB**:

   Thaw DAB and filter with syringe filter.

Glucose Oxydase	**10 μl**
Ammonium Chloride	**80 μl**
Nickel Ammonium Sulfate	**700 μl**

   Put **5 ml** of NI DAB in each vial with sections (5 sections in vial)

   Immediately add βD-Glucose (**100 μl for 5ml of NI-DAB**) and develop reaction on shaker under thorough microscopic control:

   RESULT: Neurons should be stained blue black and their axons should be well labeled with very mild grayish background.

   REAGENTS

PB	0.1M Phosphate buffer
BS - Blocking serum in PB (100ml):	NGS (10%) 10ml BSA (2%) 2ml Triton (0.4%) 4ml of 10% Triton
ABC:	1 drop of Avidin and 1 drop of Biotin (from ABC Elite Kit) for each 2.5 ml of 1% BSA. Mix at RT and let it stay on slow rotation for 30 min prior to use it on sections
DAB solution:	**20 ml 0.**05% DAB (10 mg DAB in 20 ml 0.1 mM PB) add followings: **80 ul of 10% Ammonium Chloride (1 g in 10 ml ddw)** **400 ul 2% Nickel ammonium sulfate (2 g in 100 ml ddw)** **10 ul of glucose oxidase (8 mg in 1 ml ddw, then aliquoted in 10 ul/ea)** **Pre-incubate the sections for 5-10 min, then add 400 ul 10% beta-D glucose (1 g in 10 ml ddw, then aliquoted in 400 ul/ea)**